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<p>(54) Title: GLUCOSAMINE FATTY ACID COMPOSITIONS AND THEIR USE</p> <p>(57) Abstract</p> <p>Compositions of <u>glucosamine</u> and an <u>essential fatty acid</u>, especially one or more of the "delta-6-desaturated" n-6 and n-3 essential fatty acids such as GLA, <u>DGLA</u>, <u>SA</u>, <u>EPA</u>, and <u>DHA</u>, other than compositions comprising <u>chondroitin sulphate</u>; and their use in inflammatory joint conditions including osteoarthritis and arthritis.</p> <p style="text-align: right;">P4</p>		

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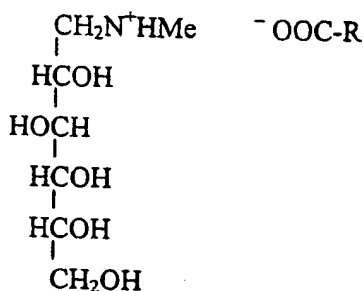
## GLUCOSAMINE FATTY ACID COMPOSITIONS AND THEIR USE

## FIELD OF INVENTION

The invention relates to the presentation and use of sugar amines.

## PRIOR PATENT

The applicants have previously filed on sugar amine salts in PCT GB 96/00952 (WO 96/33155), where N-methyl glucamine (meglumine), as an example of compounds broadly presented as N-alkyl polyhydroxy amines, is disclosed in the form of salts with polyunsaturated fatty acids. The fatty acids are particularly the "6-desaturated" n-6 and n-3 essential fatty acids i.e. those beyond the 6-desaturation stage in bodily conversion of dietary linoleic and  $\alpha$ -linolenic acids, and may be as such or in the form of their esters or amides with a bifunctional compound also having a salt-forming acidic function. Examples of such compounds are ascorbic acid, when the fatty acid is as a 6-ester, and salicylic acid. The N-methyl glucamine salts are:-



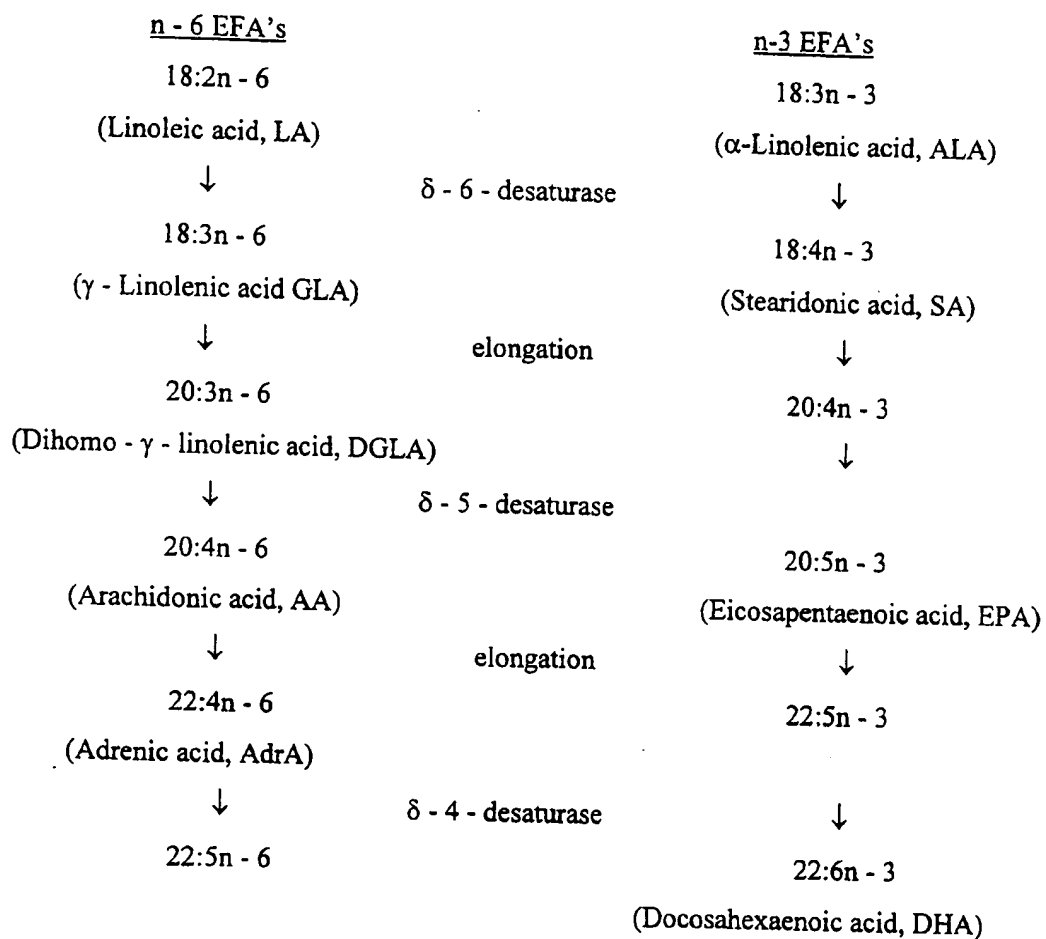
where R is the carbon chain of the fatty acid.

The purpose is to present the fatty acids in water soluble form for their numerous therapeutic and other actions, allowing for example ready absorption from the gut into the hepatic portal system, or intravenous administration. However the meglumine moiety, lacking a carbonyl function, is only formally related to sugars and has no function other than as a carrier of the fatty acid.

## FATTY ACIDS

The essential fatty acids (EFAs) and their bodily conversion pathways are set out in Table 1 below.

TABLE 1



The acids, which in nature are of the all - cis configuration, are systematically named as derivatives of the corresponding octadecanoic, eicosanoic or docosanoic acids, e.g. z,z-octadeca - 9,12 - dienoic acid for LA or z,z,z,z,z,z - docosa- 4,7,10,13,16,19 - hexaenoic acid for DHA, but numerical designations based on the number of carbon atoms, the number

of centres of unsaturation and the number of carbon atoms from the end of the chain to where the unsaturation begins, such as, correspondingly, 18:2 n-6 or 22:6 n-3, are convenient.

Initials, e.g. EPA and shortened forms of the name e.g. eicosapentaenoic acid are used as trivial names in some of the cases. The acids beyond the delta-6-desaturation stage are sometimes referred to as the delta-6-desaturated acids.

## GENERAL BACKGROUND

There has been much general attention to the maintenance of healthy cartilage and bones; to prevention of cartilage degeneration as part of the normal ageing process or as a result of disease; and to treatment of inflammation and pain associated with disease of the joints, especially in osteoarthritis and rheumatoid arthritis, and also including degeneration and inflammation of the cartilage of the vertebral column.

Drug treatment. While analgesics (e.g. paracetamol and codeine) and non-steroidal anti-inflammatory drugs (NSAIDs) are much used for relief of pain associated with osteoarthritis, rheumatoid arthritis and back problems, the NSAIDs are limited by their side effects and there is some evidence that they may actually impair cartilage metabolism. Other pharmacological approaches include intra-articular steroid therapy, mechanical lubrication and chondroprotective agents including D-glucosamine sulphate, glycosaminoglycan-peptide complex and glycosaminoglycan polysulphate.

As regards fatty acids, ingestion of for example GLA (e.g. from evening primrose oil) elevates DGLA levels which results in an increase in production of anti-inflammatory 1-series prostaglandins such as PGE1. Ingested EPA (e.g. from fish oil) provides a substrate for production of anti-inflammatory 3-series prostaglandins and 5-series leukotrienes. The anti-inflammatory activity of both GLA and EPA in animal and human studies is well documented in prior patents of the applicants and in the general literature.

As a structural element, the amino-monosaccharide glucosamine is found in the matrix of articular cartilage where it is a component of various glycosaminoglycans.

Synthesis of glycosaminoglycans requires sulphate ions as well as glucosamine. *In vitro* studies have long demonstrated that glucosamine stimulates the uptake of radioactively labelled sulphate ions by chondrocytes as a result of glycosaminoglycan synthesis. More recent *in vitro* studies have supported the conclusion that glucosamine stimulates synthesis of glycosaminoglycan and proteoglycans in general in chondrocytes.

Additional properties of glucosamine include protection of articular cartilage from damage caused by some non-steroidal anti-inflammatory drugs as well as anti-inflammatory activity in models of acute and sub-acute inflammation not caused by inhibition of prostaglandin synthesis. Also, several clinical trials have demonstrated the efficacy of glucosamine in the control of symptoms of osteoarthritis. It has been concluded that oral glucosamine is as effective as a standard NSAID such as ibuprofen, in controlling symptoms with clinically evident signs of inflammation, while it is significantly better tolerated.

In considering this background it may seem surprising that no suggestion has been made of combining the effects of fatty acids and amino-sugars in relation to cartilage and bone disorders and especially osteoarthritis or other disorders of the joints. Nevertheless that appears to be so, and although the individual use of both glucosamine and long chain polyunsaturated fatty acids is known, the admixtures and particularly the novel chemical entities described herein give significant advantages, over current treatments. Part of the reason for this is undoubtedly the fact that lipophilic compounds like the fatty acids and hydrophilic compounds like glucosamine have not traditionally been combined within a single molecule.

## THE INVENTION

The invention concentrates on glucosamine as a natural cartilage constituent and in chemical terms provides:-

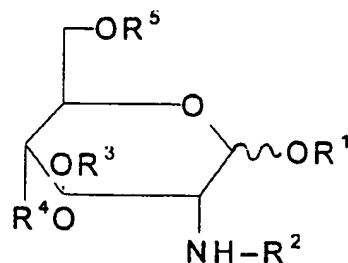
Firstly: novel compositions of glucosamine and essential fatty acids, especially the delta-6-desaturated n-6 and n-3 essential fatty acids such as GLA and EPA. The fatty acids

may be used in natural form e.g. as evening primrose oil, fish oil, fungal oil, algal oil or other assimilable forms, or alternatively they may for example be present as salts of the glucosamine itself.

Secondly: novel chemical entities combining the glucosamine and the fatty acids.

More particularly, in terms of novel chemical entities, the invention provides amide, ester or amide/ester derivatives of glucosamine wherein one or more of the amino and hydroxy functions carries a fatty acyl group derived from an n-6 or n-3 essential fatty acid, the fatty acid being linked to the amine or hydroxy function either directly or through a linking compound itself having a carboxylic acid function and in the latter case being replaceable by a corresponding fatty alcohol group esterified to a second carboxylic acid function of the linking compound.

Such a structure may be as set out below:-



wherein:-

- i)  $R^1$  to  $R^5$  are independently H or a  $\text{UFA}-\text{C}(=\text{O})$  or  $\text{UFA}^1-\text{C}(=\text{O})$  group the same or different (but not all H) with the further possibility (when at least one of  $R^1$ ,  $R^3$ ,  $R^4$  and  $R^5$  is such a group) that  $R^2$  is a  $\text{C}_1$  to  $\text{C}_4$  acyl group or other biocompatible derivatising function;

- ii) UFA is the carbon chain of an n-6 or n-3 preferably 6-desaturated essential fatty acid, and very preferably a fatty acid with anti-inflammatory activity such as GLA, DGLA, stearidonic acid, EPA or DHA;
- iii) UFA<sup>1</sup> is the carbon chain of such a fatty acid, or of a corresponding fatty alcohol, covalently linked to a biocompatible bifunctional compound also having a free acidic group;

and wherein when  $R^2 = H$ , the compound may be in the form of the free base or as a salt with a pharmaceutically acceptable acid

In terms of new uses the invention lies in application of compounds or compositions as above in the therapeutic or prophylactic treatment of disease or deterioration in bodily cartilaginous tissue. The cartilaginous tissue may in particular be that of the joints including those of the vertebral column and the invention is particularly applicable in inflammatory joint conditions including osteoarthritis, other forms of arthritis and conditions leading to back pain.

In both aspects of the invention the amounts of the fatty acids for daily administration are 10 mg to 10 g preferably 100 mg to 5 g and very preferably 300 mg to 3g and of the sugar amines 10 mg to 20 g, preferably 100 mg to 5 g and very preferably 300 mg to 3 g, preferably in corresponding molar amounts to the fatty acid where the two are not chemically combined. The compounds may be applied by oral, enteral, parenteral or topical routes. The compounds may be used directly in liquid form, or be formulated as emulsions, powders, tablets, or hard or soft gelatin capsules or in any other appropriate form known to those skilled in the art.

The invention is illustrated by the following synthesis and application examples.



## SYNTHETIC ROUTES

Synthesis of the sugar amine derivatives will require the formation of ester and/or amide bonds.

1. Any reasonable method of ester synthesis may be applied to glucosamine itself or suitable N-derivatives, especially:

(i) by reaction of the glucosamine or derivative with acid chloride, acid anhydride or suitably activated ester, e.g. 2,4-dinitrophenol ester, with or without a suitable organic tertiary base, e.g. pyridine, in a suitable inert solvent, e.g. dichloromethane, and at a temperature between 0° and 120°C.

(ii) by reaction of the glucosamine or derivative with acid, or acid short or medium chain alkyl ester, in the presence of a suitable acid catalyst, e.g. 4-toluene sulphonic acid, with or without a suitable inert solvent, e.g. toluene, at a temperature between 50° and 180°C, such that the water or alcohol formed in the reaction is removed, e.g. by azeotropy or under vacuum.

(iii) by reaction of glucosamine or derivative with acid in the presence of a condensing agent, e.g. 1,3-dicyclohexylcarbodiimide, with or without a suitable organic base e.g. 4-(N,N-dimethylamino)pyridine in an inert solvent, e.g. dichloromethane, at a temperature between 0° and 50°C.

(iv) by reaction of glucosamine or derivative with acid, or acid short or medium chain alkyl ester or activated ester e.g. vinyl, in the presence of a hydrolase enzyme, e.g. Novozym-435<sup>TM</sup>, with or without a suitable solvent, e.g. *tert*-butanol, at temperatures between 20° and 80°C under conditions such that the water or alcohol byproduct formed in the reaction is removed from the reaction mixture, e.g. by molecular sieves or under vacuum.

(v) by reaction of acid with a suitable glucosamine derivative, e.g. tosylate, with or without the presence of a suitable base, e.g. potassium carbonate, in a suitable inert solvent, e.g. dimethylformamide, and at a temperature between 0° and 180°C.

(iv) by reaction of acid ester (UFA-COOY) with the glucosamine or derivative in the presence of a catalytic amount of an alkoxide of type  $M^+OY^-$  where M is an alkali or alkaline earth metal, e.g. sodium, and Y is an alkyl group containing 1-4 carbon atoms which may be branched or unbranched and saturated or unsaturated. The reaction is carried out with or without a suitable solvent, e.g. toluene, at temperatures between 50° and 180°C such that the lower alcohol, HOY, formed is removed from the reaction mixture, e.g. by azeotropy or under vacuum.

2. Any reasonable method of amide synthesis may be applied and especially:

(i) by reaction of the glucosamine or derivative with acid chloride, acid anhydride or suitably activated ester, e.g. N-hydroxysuccinimide, with or without the presence of an organic tertiary base, e.g. pyridine, in a suitable inert solvent, e.g. dichloromethane, and at a temperature between 0° and 120°C.

(ii) by reaction of the glucosamine or derivative with acid in the presence of a condensing agent e.g. 1,3-dicyclohexylcarbodiimide with or without a suitable organic base, e.g. 4-(N,N-dimethylamino)pyridine, in an inert solvent, e.g. dichloromethane, at a temperature between 0° and 50°C.

(iii) by reaction of the glucosamine or derivative e.g. N-acetyl or N-t-BOC (butoxycarbonyl) with acid, or acid short or medium chain alkyl ester or activated ester, e.g. vinyl, in the presence of a hydrolase enzyme, e.g. Novozym-435<sup>TM</sup>, with or without a suitable solvent, e.g. *tert*-butanol, at temperatures between 20° and 80°C under conditions such that the water or alcohol byproduct formed in the reaction is removed from the reaction mixture, e.g. molecular sieves or under vacuum.

## SPECIFIC SYNTHESSES AND FORMULATIONS

The invention is illustrated by way of example in the following:

**Example 1**

N-(z,z,z-octadeca-6,9,12-trienoyl)-D-glucosamide

(Synthesis of amide of GLA with glucosamine)

Part 1 - preparation of z,z,z-octadeca-6,9,12-trienoic acid, N-hydroxysuccinimide ester

A solution of z,z,z-octadeca-6,9,12-trienoyl chloride in methylene chloride was added dropwise to a cooled solution of N-hydroxysuccinimide and triethylamine in methylene chloride. The reaction was stirred for a further hour after addition was complete. TLC analysis indicated complete reaction. The reaction mixture was poured into hexane, filtered and concentrated to yield z,z,z-octadeca-6,9,12-trienoic acid, N-hydroxysuccinimide ester as a pale yellow, fairly viscous oil.

Part 2 - preparation of N-(z,z,z-octadeca-6,9,12-trienoyl)-D-glucosamide

A solution of z,z,z-octadeca-6,9,12-trienoic acid, N-hydroxysuccinimide ester in THF (tetrahydrofuran) was added to a solution of D-glucosamine and sodium hydrogen carbonate in water at room temperature and the resulting mixture was stirred overnight under nitrogen. A layer of yellow oil which had formed at the base of the flask was removed. Analysis of this oil indicated that it was not the desired product. The remaining solution was concentrated under reduced pressure to remove the bulk of the THF. A dense white solid formed. Water was added and the solid recovered by centrifugation. This solid was washed with water and dried under high vacuum to yield N-(z,z,z-octadeca-6,9,12-trienoyl)-D-glucosamide as an off-white amorphous solid.

**Example 2**

N-(z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoyl)-D-glucosamide  
(Synthesis of amide of EPA with glucosamine)

In a similar manner to Example 1 but replacing z,z,z-octadeca-6,9,12-trienoyl chloride with z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoic acid was prepared N-(z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoyl)-D-glucosamide as an off-white amorphous solid.

**Example 3**

(Preparation of administration forms comprising the above amides)

The products of Examples 1 and 2 may be used without further purification in preparations for the purposes referred to herein, for example the treatment of osteoarthritis:-

1. Sterile solutions for topical or local administration containing 0.1 - 20% by weight of the product.
2. Oral pharmaceutical preparations containing 100 mg to 1 g, in 5 ml, of the product.
3. Sterile pharmaceutical solutions for intravenous administration containing 0.1 to 20% by weight of the product.

**Example 4**

(Capsules of glucosamine as a salt and fatty acids in natural form).

Soft gelatin capsules conventional in themselves contain:-

295 mg evening primrose oil (8% GLA)

73 mg marine fish oil (8% EPA, 12% DHA)

250 mg D-glucosamine sulphate.2NaCl

15 mg tocopheryl acetate antioxidant

and the capsules are administered six capsules per day for up to 12 weeks then 2 capsules per day, to combat osteoarthritis.

### Example 5

1-((3-(z,z,z-octadeca-6,9,12-trienoyloxy)propyl)oxycarbonyl)-4-butanoyl-D-glucosamide  
(Synthesis of succinic acid, amide with D-glucosamine, ester with 3-hydroxypropyl ester of GLA)

#### Part 1: preparation of 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-3-hydroxypropane

A solution of z,z,z-octadeca-6,9,12-trienoic acid (150g) in methylene chloride (500ml) was added dropwise to a mixture of 1,3-propane diol (205g), 1,3-dicyclohexylcarbodiimide (130g) and 4-(N,N-dimethylamino)pyridine (87g) in methylene chloride (2.5l) at room temperature under nitrogen. When tlc indicated that the reaction had gone to completion, the reaction mixture was filtered. The filtrate was washed with dilute hydrochloric acid, water and saturated sodium chloride solution. The solution was dried, concentrated and purified by dry column chromatography to yield 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-3-hydroxypropane as a pale yellow oil.

#### Part 2: preparation of butanedioic acid, monoester with 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-3-hydroxypropane

A mixture of 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-3-hydroxypropane (10g) and succinic anhydride (3g) in dry DMF (100ml) was stirred at room temperature until a clear solution resulted. This solution was cooled to 0°C and a solution of 1,8-diazabicyclo[5.4.0]undec-7-ene (4.5ml) in dry THF (50ml) added dropwise to it. After 3h, tlc analysis indicated that most of the monoester had reacted. A few more crystals of succinic anhydride were added and stirring continued for a further 30 min. The reaction mixture was diluted with diethyl ether (250ml), washed with 2M hydrochloric acid (2 x 250ml), water (250ml) and brine (250ml), dried (sodium sulphate) and concentrated to dryness. The material was used without further purification.

Part 3: preparation of 1-(3-(z,z,z-octadeca-6,9,12-trienoyloxy)propyl)oxycarbonyl-4-butanoyl chloride

Oxalyl chloride (3.9ml) was added to a solution of butanedioic acid, monoester with 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-3-hydroxypropane (13g) in methylene chloride (75ml). The mixture was stirred at room temperature under nitrogen for 2h and concentrated to dryness. Hexane (75ml) was added and the mixture concentrated to dryness. This process was repeated with two further portions of hexane to yield 1-(3-(z,z,z-octadeca-6,9,12-trienoyloxy)propyl)oxycarbonyl-4-butanoyl chloride as a pale yellow oil.

Part 4: preparation of 1-(3-(z,z,z-octadeca-6,9,12-trienoyloxy)propyl)oxycarbonyl-4-butanoyl acid, N-hydroxysuccinimide ester

A solution of 1-(3-(z,z,z-octadeca-6,9,12-trienoyloxy)propyl)oxycarbonyl-4-butanoyl chloride in methylene chloride was added dropwise to a cooled solution of N-hydroxysuccinimide and triethylamine in methylene chloride. The reaction was stirred for an hour after the addition was complete. Tlc analysis indicated complete reaction. The reaction mixture was poured into hexane, filtered and concentrated to yield the ester as a pale yellow oil.

Part 5: preparation of 1-((3-(z,z,z-octadeca-6,9,12-trienoyloxy)propyl)oxycarbonyl)-4-butanoyl-D-glucosamide

A solution of 1-(3-(z,z,z-octadeca-6,9,12-trienoyloxy)propyl)oxycarbonyl-4-butanoyl acid, N-hydroxysuccinimide ester in THF was added to a solution of D-glucosamine and sodium hydrogen carbonate in water at room temperature and the resulting mixture was stirred overnight under nitrogen. A layer of yellow oil at the bottom of the flask was discarded and the remaining solution was concentrated under reduced pressure. Addition of water precipitated a solid which was recovered by centrifugation. This solid was washed with water and dried under high vacuum to yield 1-((3-(z,z,z-octadeca-6,9,12-trienoyloxy)propyl)oxycarbonyl)-4-butanoyl-D-glucosamide as an off-white amorphous solid.

**Example 6**

1-(z,z,z-octadeca-6,9,12-trienyloxycarbonyl)-4-butanoyl-D-glucosamide  
(Synthesis of succinic acid, amide with D-glucosamine, ester with GLA alcohol)

In a similar manner to Example 5 but replacing 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-3-hydroxypropane in Part 2 with z,z,z-octadeca-6,9,12-trienol (prepared by reduction of z,z,z-octadeca-6,9,12-trienoic acid) was prepared 1-(z,z,z-octadeca-6,9,12-trienyloxycarbonyl)-4-butanoyl-D-glucosamide as an off-white amorphous solid.

**Example 7**

Glucosammonium z,z,z-octadeca-6,9,12-trienoate  
(Synthesis of glucosamine salt of GLA).

z,z,z-Octadeca-6,9,12-trienoic acid (2.78g) is added dropwise with stirring under nitrogen to a solution of D-glucosamine (1.79g) in water (50 ml). Stirring is continued at room temperature until a clear solution results. Lyophilisation yields glucosammonium z,z,z-octadeca-6,9,12-trienoate.

The products of Examples 5 to 7 may be used in the manner of Examples 3 and 4.

**Disclaimer**

Published PCT specification WO 97/21434 (19 June 1997) gives e.g. At page 11 para. 1 a composition of N-acetyl glucosamine sulphate, glucosamine sulphate and chondroitin sulphate, as "CHONDROX", to be used with EFAs, against arthritis and specifically osteo Arthritis (The glucosamine and fatty acids are not as mutual salts or otherwise in combination). The broad compositions claims herein thus do not extend to compositions comprising chondroitin sulphate as such or as "CHONDROX".

CLAIMS

1. Compositions of glucosamine and an essential fatty acid, especially one or more of the "delta-6-desaturated" n-6 and n-3 essential fatty acids such as GLA, DGLA, SA, EPA and DHA, other than compositions comprising chondroitin sulphate.
2. Glucosamine and an essential fatty acid (especially as listed in claim 1) when in combination covalently or as a mutual salt, particularly an amide, ester or amide-ester derivative of glucosamine wherein one or more of the amino and hydroxy functions carries a fatty acyl group derived from an n-6 or n-3 essential fatty acid, the fatty acid being linked to the amine or hydroxy function either directly or through a linking compound itself having a carboxylic acid function and in the latter case being replaceable by a corresponding fatty alcohol group esterified to a second carboxylic acid function of the linking compound.
3. Use of an essential fatty acid and (especially as listed in claim 1) and of glucosamine, optionally in combination as in claim 2, or of one of them when the other is to be administered separately, in the preparation of a medicament for therapeutic or prophylactic treatment of disease or deterioration in bodily cartilaginous tissue in particular that of the joints including those of the vertebral column, more particularly in inflammatory joint conditions including osteoarthritis, other forms of arthritis and conditions leading to back pain, and such treatment itself.
4. The subject of any preceding claim, the amounts of the fatty acid for daily administration being 10 mg to 10 g preferably 100 mg to 5 g and very preferably 300 mg to 3g and of the glucosamine 10 mg to 20 g, preferably 100 mg to 5 g and more preferably 300 mg to 3g, preferably further in corresponding molar amounts to the fatty acid where the two are not combined.



# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 98/01425

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K31/205 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 01645 A (PEPTECH UK LTD ; LEDGER PHILIP (GB)) 25 January 1996 see page 12, line 16 - page 19, line 28; claims 3,7,8,10,19 ---	1-4
X,P	WO 97 21434 A (INHOLTRA INC ; FLORIO VITO V (US)) 19 June 1997 cited in the application see abstract see page 8, line 21 - page 8, line 25; claims 1-4 see page 10, line 1 - page 13, line 29 --- -/--	1-4

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

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Date of the actual completion of the international search

6 October 1998

Date of mailing of the international search report

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A. Jakobs

# INTERNATIONAL SEARCH REPORT

Intern. Application No

PCT/GB 98/01425

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE WPI  Section Ch, Week 8415  Derwent Publications Ltd., London, GB;  Class D21, AN 84-092340  XP002078424  &amp; JP 59 039813 A (SHISEIDO CO LTD)  , 5 March 1984  see abstract</p> <p style="text-align: center;">---</p>	1,2
X	<p>MILLER ET AL.: "Studies of the  Specificity and Cross-Reactions of  Antibodies to Lipid A Found in Juvenile  Arthritis"  CLIN. DIAGN. LAB. IMMUNOL.,  vol. 2, no. 2, 1995, pages 186-191,  XP002078420</p>	1,2
A	<p>see abstract; figure 1; table 1</p> <p style="text-align: center;">---</p>	3,4
X,P	<p>CHEMICAL ABSTRACTS, vol. 127, no. 20,  17 November 1997  Columbus, Ohio, US;  abstract no. 274048,  CHOI, BYEONG-DAE ET AL: "Quality  improvement of rainbow trout with pigments  and enzymic hydrolyzates of ascidian  (Halocynthia roretzi) tunic. 1. Chemical  specificity of ascidian tunic and its  hydrolyzates"  XP002078423  see abstract</p>	1,2
X	<p>&amp; CHOI, BYEONG-DAE ET AL: "Quality  improvement of rainbow trout with pigments  and enzymic hydrolyzates of ascidian  (Halocynthia roretzi) tunic. 1. Chemical  specificity of ascidian tunic and its  hydrolyzates"  HAN'GUK SUSAN HAKHOECHI (1996), 29(3),  345-356 CODEN: HSHKAW;ISSN: 0374-8111,</p> <p style="text-align: center;">---</p>	1,2
X,P	<p>PHILIP-HOLLINGSWORTH ET AL.: "Structural  requirements of Rhizobium  chitolipooligosaccharides for uptake and  bioactivity in legume roots as revealed by  synthetic analogs and fluorescent probes"  J. LIPID RESEARCH,  vol. 38, no. 6, 1997, pages 1229-1241,  XP002078421  see page 1231, column 1, paragraph 2 -  page 1233, column 2, paragraph 2; figures  1-3; table 1</p> <p style="text-align: center;">---</p>	1,2
P,X	<p>US 5 639 787 A (RIORDAN ET AL.)  17 June 1997  see claims 9,10</p> <p style="text-align: center;">---</p>	1,2
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# INTERNATIONAL SEARCH REPORT

Intern: al Application No

PCT/GB 98/01425

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BUDAVAARI ET AL.: "The Merck Index" 1989 , MERCK & CO. , INC. , RAHWAY, N.J., USA XP002078422 19996 see page 4353, column 2, paragraph 3-5 ---	1-4
A	WO 93 24505 A (ALBERTA RES COUNCIL ;IPPOLITO ROBERT M (CA); HAQUE WASIMUL (CA); H) 9 December 1993 see page 26, line 32 - page 27, line 3; examples 1-53 ---	1-4
A	WO 96 33155 A (SCOTIA HOLDINGS PLC ;HORROBIN DAVID F (GB); KNOWLES PHILIP (GB); M) 24 October 1996 see abstract; claims 1-10; examples 1-5 see examples 1-5 -----	1,2

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 98/ 01425

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International Application No. PCT/ GB 98 /01425

### FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

In view of the large number of compounds which are defined by the general definitions/formulae used in claims 1-4, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and / or the compounds mentioned in the claims, and to the general idea underlying the application. (see Guidelines, chapter III, paragraph 2.3).

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 98/01425

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Information on patent family members

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